

corpuscular hemoglobin concentration, among animals (female and male) from the high and intermediate dose groups. Lymphocyte counts were also reduced (up to 50%) among the high dose treated animals. Generally, effects noted among the female animals reached statistical significance ($p < 0.05$), while those changes seen in the affected male animals were marginal or not statistically significant. Similar, although somewhat smaller, effects were seen among the high dose treated animals at the conclusion of the 26 week drug administration interval.

Consistent changes in serum chemistry noted during the dosing interval included: a) a slight reduction in serum inorganic phosphorus levels (all drug treated animals but especially evident among treated females), b) significant reduction in serum calcium levels among the high dose treated female animals, c) a slight decrease in serum protein, albumin or globulin concentration (sporadically significant reductions evident among the high and intermediate dosed animals), d) a slight dose related increase in serum cholesterol levels (primarily evident among female animals; the effect occasionally being statistically significant), e) a slight dose related reduction in serum ALT levels (statistical significance on sporadic occasions), and f) a slight elongation of the partial thromboplastin time among the high dose treated animals.

No other changes in hematologic or serum chemistry parameters were noted as drug related among the treated animals.

Gross and Microscopic Pathology: At necropsy, decreases in both the absolute and relative weights of the thymus were noted in the majority of the drug treated animals. The changes in thymic weight were generally dose related. Microscopic examination of multiple lymphoid tissues revealed some evidence of disseminated lymphoid atrophy in the spleen, thymus, and lymph nodes of the majority of the high dose treated animals.

Other abnormalities noted at necropsy or on histologic evaluation included: a) a slightly increased incidence of nodules in cardiac and pulmonary tissues of the drug treated animals, and b) immaturity of the ovaries (lack of mature follicles) among the high dose treated female animals. All remaining gross and/or microscopic lesions appeared to be randomly distributed among the treatment and control groups.

Results After 52 Weeks of Ribavirin Administration

Mortality: One male animal from the intermediate dose treatment group (10 mg/kg/day) was found dead during week 49 of the study following a period of decreased food consumption and weight loss. Similar to the findings in the other precedent animal, the post-mortem examination revealed fluid accumulation in the lungs, inflammatory changes and evidence of pneumonia (potentially suggestive of a gavage accident). The remaining study animals were all terminated at the conclusion of the treatment interval.

Clinical Signs: Similar to findings during the initial 26 weeks of ribavirin treatment, there was a slight increase in the incidence of loose and/or bloody stools among several male and female animals from the high dose treatment group. Post-dose vomiting was also noted among male and female animals from the high dose group (i.e., 20 mg/kg/day), although the incidence was only slightly higher than for the concurrent controls. There were no other clinical signs which appeared related to the test compound.

Body Weight and Food Consumption: Body weight & weight gain were slightly decreased (mean reductions of approximately 5% and 20-30%, respectively) among male and female animals dosed at 20 mg/kg/day. The reduction in weight gain and/or body weight was generally evident within 1 week of the initiation of

dosing. Males and females in the intermediate dose group also gained less weight throughout or during the early phase of drug treatment (male and female animals, respectively), although the mean body weight of these animals was not statistically different from the control at the conclusion of dosing. Food consumption was reduced (approx. 5-30%) among the high dose treated animals, although the effect only occasionally achieved statistical significance among the high dose treated female animals. The weight gain and food consumption of animals in the control and low dose groups were comparable throughout the dosing interval.

Cardiac Examinations: There were no apparent drug related effects on any cardiac parameter noted among the drug treated animals.

Ophthalmoscopy: Except as discussed in the preceding section (related to findings after 26 weeks of drug administration), there were no apparent drug related effects on any ophthalmologic abnormalities detected.

Hematology and Clinical Chemistry: As discussed in the preceding section, there was evidence of slight to moderate reductions (5-30%) in mean hemoglobin, RBC counts, hematocrit, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, among animals (female and male) from the high and intermediate dose groups. Lymphocyte, eosinophil and monocyte counts were also reduced sporadically (up to 50%) among the high dose treated animals. Generally, the effects noted among the high dose treated male animals remained evident throughout the 52 week dosing interval, while the initial adverse hematologic effects noted among the high dose treated female animals showed some evidence of recovery with continued drug administration. There was no clear evidence of a change in the cellularity of the bone marrow following either 26 or 52 weeks of ribavirin dosing.

Changes in serum chemistry included: a) a slight reduction in serum inorganic phosphorus levels among the high dose treated female animals (occasionally also seen among the high dose treated male animals), b) reductions in serum calcium levels among the high dose treated female animals, c) a slight decrease in serum protein, albumin and/or globulin levels among the high and intermediate dose treated animals, d) a slight dose related increase in serum cholesterol levels during the early phase of drug treatment and followed by significant reductions in serum cholesterol levels (evident primarily among the high dose treated animals), e) periodic dose related reductions and increases in serum ALT and AST levels among the high and intermediate dose treated animals (primarily evident among male animals; the fluctuations occasionally achieving statistical significance), and f) a slight elongation of the partial thromboplastin time among the high dose treated animals.

There were no other changes in any hematologic or serum chemistry parameters which appeared related to ribavirin treatment.

Drug Exposure: The study data indicate that the mean assayed dosing preparations were within 10% of the nominal doses. Plasma and erythrocyte drug levels were measured in in-life (1 hour after dosing) and necropsy specimens and are reported in the following tables.

Comments: 1) The data suggest that in the dog, ribavirin is well absorbed after oral administration and plasma drug levels generally increase in an approximately linear manner with the nominal dose. The data also suggest that a slight increase in plasma drug levels may occur over an extended period of drug exposure.

Dose (mg/kg)		Plasma Drug Levels (μMolar)			
		0	5	10	20
Sex					
♂	WK1	BLD	4.36	7.99	14.65
	WK2	BLD	4.34	9.25	16.11
	WK3	BLD	3.84	9.55	18.44
	WK4	BLD	4.21	9.51	17.50
	WK8	BLD	4.83	9.94	16.70
	WK13	BLD	4.93	10.90	18.12
	WK18	BLD	5.04	10.57	16.23
	WK22	BLD	5.44	10.86	18.52
	WK26	BLD	5.41	11.09	20.80
	WK30	BLD	4.45	9.55	21.63
	WK34	BLD	3.35	7.75	24.78
	WK39	BLD	3.77	8.85	23.60
	WK44	BLD	3.90	10.05	24.15
	WK48	BLD	3.98	9.80	24.00
	WK52	BLD	6.93	11.03	25.23
♀	WK1	BLD	3.89	8.75	15.76
	WK2	BLD	4.41	8.05	16.94
	WK3	BLD	4.24	8.46	18.29
	WK4	BLD	3.88	8.75	17.24
	WK8	BLD	4.85	8.91	15.95
	WK13	BLD	4.59	9.09	16.58
	WK18	BLD	4.58	8.95	17.56
	WK22	BLD	4.71	10.09	18.58
	WK26	BLD	4.39	9.26	19.44
	WK30	BLD	3.70	8.28	23.93
	WK34	BLD	3.63	7.05	23.35
	WK39	BLD	3.45	7.20	23.60
	WK44	BLD	4.05	8.83	23.30
	WK48	BLD	3.98	8.93	24.75
	WK52	BLD	5.88	10.18	24.40

BLD = Below Limit of Detection (LD = 0.025 μMoles in Plasma)

Dose (mg/kg)		Erythrocyte Drug Levels (μMolar)			
		0	5	10	20
Sex					
♂	WK1	BLD	5.00	9.56	10.88
	WK2	BLD	8.81	20.12	16.46
	WK3	BLD	9.68	10.83	24.41
	WK4	BLD	10.44	13.76	20.73
	WK8	BLD	14.80	13.56	25.58
	WK13	BLD	14.06	16.89	28.86
	WK18	BLD	12.11	15.21	24.00
	WK22	BLD	15.33	25.40	28.73
	WK26	BLD	11.54	16.68	21.20
	WK30	BLD	15.83	22.23	35.00
	WK34	BLD	16.85	22.98	33.28
	WK39	BLD	12.84	19.68	28.55
	WK44	BLD	15.05	23.50	31.60
	WK48	BLD	18.50	26.08	35.40
	WK52	BLD	16.38	21.33	29.58

Dose (mg/kg)		Erythrocyte Drug Levels (μMolar) (cont.)			
		0	5	10	20
♀	WK1	BLD	4.43	9.27	12.07
	WK2	BLD	7.17	24.05	21.95
	WK3	BLD	8.23	11.71	23.54
	WK4	BLD	8.34	13.00	21.00
	WK8	BLD	14.38	14.33	27.56
	WK13	BLD	11.64	18.48	35.36
	WK18	BLD	10.95	15.88	30.50
	WK22	BLD	12.39	25.80	30.05
	WK26	BLD	10.12	12.22	22.67
	WK30	BLD	14.38	22.33	28.80
	WK34	BLD	15.23	26.75	35.08
	WK39	BLD	14.15	25.55	33.78
	WK44	BLD	15.48	26.13	27.55
	WK48	BLD	17.45	27.38	31.55
	WK52	BLD	16.73	22.23	29.48

BLD = Below Limit of Detection (LD = 2.0 μMoles in RBC)

Comments (cont.):

2) Unlike the data for plasma drug levels, the erythrocyte content of ribavirin (presumably the tri-phosphate form) appears to have increased continuously during the initial 26-30 weeks of dosing, and remained relatively stable at these increased levels for the remainder of the dosing interval. The significance of these finding relative to the hemolytic and/or anemia inducing effects of ribavirin is not known.

Gross and Microscopic Pathology: Unlike the findings noted after 26 weeks of ribavirin exposure (i.e., gross thymic atrophy and microscopic lymphoid tissue depletion), there was no consistence evidence of any gross or microscopic pathology associated with exposure to ribavirin at any dose tested. All abnormalities noted at necropsy or on histologic evaluation appeared to be randomly distributed among the drug treated and control animals.

Comments: 1) As has been noted in several previously submitted toxicology studies, anemia and lymphopenia have been observed in nearly all animal species following repeat dosing with ribavirin. These effects usually become evident within 4 weeks of the initiation of ribavirin administration. In several studies, including the present, the data suggest that there may be partial recovery from the drug induced anemia during periods of continued dosing.

2) As noted in the preceding sections, administration of ribavirin was associated with slight reductions in serum proteins, albumin and ALT levels, among animals dosed at 20 mg/kg/day (smaller effects were sometimes evident among animals dosed at lower levels). No histo-logic abnormalities were evident in either the liver or kidneys to account for these changes. The data suggest that the changes in serum protein/albumin and ALT might be the result of decreased synthesis. Thus, while serum protein/albumin and transaminase levels generally remained near-normal throughout treatment, the observed decreases in serum protein/albumin and ALT levels may have resulted from a ribavirin induced inhibition of liver synthetic capacity.

3) The results of this study suggest that 5 mg/kg/day may be

considered the NOEL dose for ribavirin when administered orally to the beagle dog for 6 months.

4) The suppression of circulating white blood cells was accompanied by evidence of lymphoid tissue depletion and atrophy (loss of germinal centers, macrophage and other inflammatory cell infiltrates, and congestion) of the thymus, spleen and lymph nodes among the affected animals after 26, but not after 52, weeks of repeated drug administration. The ribavirin induced suppression of red blood cell parameters was not associated with any microscopic evidence of compensatory changes in the bone marrow. These data, in conjunction with the results from several previously submitted studies, suggest that there may be some degree of compensatory changes and/or recovery evident in lymphoid tissue and the bone marrow during prolonged periods of ribavirin dosing.

5) The study results indicate that the concentration of ribavirin in plasma and erythrocytes increased over the initial 30 weeks of drug dosing. In general, the plasma drug levels increased gradually by a factor of 25-50%, while the intra-erythrocyte content of ribavirin went up markedly by a factor of 2-4X the concentration detected after 1 week of dosing. There were no apparent differences in plasma or erythrocyte drug levels for male and female animals.

6) As noted above, after 26 weeks the administration of ribavirin was associated with reductions in serum proteins and ALT levels, and sporadic increases in serum AST levels, among both male and female animals administered ribavirin at levels ≥ 10 mg/kg/day.

As discussed in relation to another study (conducted in the rat), no biochemical or histologic abnormalities were noted that might suggest a mechanism of increased protein/albumin loss (i.e., via the kidney), which then suggests that the changes in serum protein/albumin might be the result of decreased synthesis in the liver. Additional markers of hepatic function including serum CPK levels and mitochondrial citrate synthase or cytochrome oxidase were not measured.

MONKEY

7) One Month Subcutaneous Toxicity Study of SCH 30500 In Combination With Oral (Gavage) Dosing of Ribavirin (SCH 18908) In Cynomolgus Monkeys, Study ID P-6294 (95-57).

Status: GLP

Test Site:

Study Initiation: Aug. 1995

Test Compound: SCH 30500 and SCH 18908, Batches: 35923-029-03 and 33297-129

Doses Tested: SCH 30500 - $3105 \mu\text{g}/\text{m}^2$, QOD (every other day)

SCH 18908 - 50 and 100 mg/kg, QD

Dose Volume and Route: $16.2 \text{ ml}/\text{m}^2$ (SC) and 2.5 ml/kg (PO)

Vehicle or Control: saline and water

Species, Strain, Sex, Age, WT: σ and f cynomolgus monkeys, young adult/juvenile (age not further specified), weight range: $\sigma = 2.4\text{-}5.2 \text{ kg}$; $\text{f} = 2.3\text{-}3.8 \text{ kg}$, 3-5 animals/dose group

Test Conditions: Three groups of three animals/sex received once per day oral administration of ribavirin (50 mg/kg) or every other day subcutaneous injections of interferon ($3105 \mu\text{g}/\text{m}^2$) or, a combination of the two treatments. Two additional groups of 5 animals/sex, received either saline/water administration (control group) or interferon ($3105 \mu\text{g}/\text{m}^2$, QOD) and high dose ribavirin (100 mg/kg/day). Dosing was performed for 29-32 days, with 2 animals

each from the control and high-dose combination group being retained off-dose for a 4 week recovery period.

Mortality/morbidity, clinical signs and food consumption were measured daily. Body weight, hematology, clinical and urine chemistry were measured at baseline and weekly or bi-weekly throughout the study. EKG, blood pressure, respiration and ophthalmologic examinations were also performed during treatment.

Mortality and Morbidity: One male and one female animal from the high dose combination treatment group were sacrificed in extremis one day prior to their scheduled termination date. An additional female animal from the high dose combination treatment group was found dead during the 2nd week of the off-dose recovery period. Clinical signs noted in these animals prior to death or termination included: hypoactivity, hypothermia, decreased food consumption and body weight, diarrhea, loose stools, and dehydration. Necropsy and/or histopathologic signs included: anemia, dehydration, gastritis and colitis, and in one animal suppurative pneumonia.

Clinical Signs, Body Weight and Food Consumption: A dose related increased incidence and/or severity of diarrhea, dehydration, hypothermia, pale mucus membranes (more evident late in treatment) and decreased food intake was seen primarily among the combination drug treated animals. These signs generally first became evident during the second week of drug treatment and continued throughout the remainder of the dosing interval. Among the combination treatment animals retained off-dose, one animal died with continued clinical signs, while the remaining animal showed improvement in clinical condition by week 2 of follow-up. Body weight was reduced (approximately 0.3 kg) among the majority of animals receiving the combination drug treatment, while animals dosed with ribavirin or interferon alone demonstrated transient or no effects on body weight. Food consumption was clearly suppressed among combination drug treated animals during the early phase of treatment (beginning as early as day 3 and continuing through the start of week 3 of treatment), with sporadic reductions in food intake being evident later in treatment. A single female animal treated with ribavirin alone demonstrated similar reductions in food intake during treatment. During the off-dose follow-up period, the food consumption of all surviving animals was comparable.

Blood Pressure, EKG and Respiration: A decrease in systolic, diastolic and mean arterial blood pressure, along with a reduction in heart rate was seen among the combination drug treated animals during week 4 of dosing. A similar reduction in systolic blood pressure was also evident among the animals dosed with interferon alone. The administration of interferon and/or ribavirin had no apparent effects on either the EKG or respiratory rates of the test animals.

Ophthalmoscopy: There were no apparent drug related effects on the ophthalmologic findings noted among the study animals.

Hematology: Significant (moderate to severe) dose related reductions in total erythrocyte counts, hemoglobin concentration and hematocrit were seen in the combination drug treated animals. In general, the hematologic responses seen in the combination drug treated animals were clearly evident by the second week of drug treatment and were progressive during the remaining 2 weeks of treatment. In contrast, animals dosed with either agent alone showed only slight-mild reductions in erythrocyte counts and hemoglobin concentration, generally associated with a minimal to mild degree of reticulocytosis (particularly among the interferon treated animals). No significant evidence of compensatory reticulocytosis was seen among the combination drug treated animals during treatment, although a moderate to strong reticulocytosis was seen following the cessation of combination drug treatment. In addition to the erythroid changes, slight to mild decreases in the numbers of lymphocytes and neutrophils were evident in the interferon and combination drug treated animals (during drug treatment and the initial weeks of the off-dose recovery period). In general, all hematologic parameters had returned to baseline or were demonstrating regenerative changes by the end of the off-dose recovery period.

Clinical Chemistry and Urinalysis: All animals (male and female) dosed with interferon showed reductions in serum protein and albumin, compared to pretest and concurrent control values. The decreased albumin and total protein were generally greater (mild) in the two combination dose groups during Weeks 3-4 of treatment, but were generally comparable to concurrent control values during Week +4 (recovery). Serum globulins were also mildly decreased in the majority of combination drug treated male and female monkeys beginning at week two of treatment, but were comparable to control values by week 4 off-dose. Group mean serum calcium and phosphorus concentrations were also decreased among the interferon treated (alone or in combination with ribavirin) animals, at all assessment weeks. Compared to pretest values, serum phosphorus was minimally decreased for the majority of monkeys of both sexes during Week 4 in all the SCH 30500 dose groups (alone or in combination with ribavirin). Serum phosphorus levels in the high-dose ribavirin combination dose group was similar to or approaching (males) control values during Week 4 off-dose.

Several monkeys (No. 25M and 27M) in the high-dose combination drug treatment group displayed mild to moderate increases in ALT during Weeks 3 and/or 4. AST was mildly increased in these two males during Week 3 and moderately increased in No. 27M only during Week 4 of drug treatment. There was no histopathologic correlate to indicate hepatocellular damage in either animal.

Interferon in combination with ribavirin (low or high dose) caused a minimal to mild transient decrease in urine pH and minimal to moderate increase in urine ketones in the majority of male and female monkeys during Week 2 of treatment, but which generally recovered by week 4. Other clinical chemistry parameters which differed from pretest or concurrent control values appear to occur in a random pattern and were interpreted as incidental to drug treatment.

- Comments:
- 1) Since a portion of serum calcium is bound to albumin, the treatment induced changes in albumin may have artifactually produced the observed changes in serum calcium levels.
 - 2) The administration of interferon has previously demonstrated significant hepatotoxic potential in multiple animal species and man. However, the fact that this adverse effect was only seen in the high dose combination treatment group suggests that ribavirin may in some way be enhancing this toxic potential. However, since resolution of the elevated ALT and AST levels occurred while still on dose in one animal, and no histologic abnormality was evident in either animal, this effect may be transient or of reduced concern.
 - 3) Studies conducted in the rat and dog suggest that ribavirin may be associated with reductions in serum protein and albumin levels. Since this effect was somewhat more prominent among the combination treated animals in this study, versus the animals treated with either of the individual drugs, it should be assumed that ribavirin and interferon may be additive and/or synergistic in their inhibitory effect on circulating serum proteins (perhaps due to a drug induced decreased synthetic capacity of the liver or an increased elimination rate for these proteins).
 - 4) The increase in urine ketone concentration, the result of decreased food intake and increased gluconeogenesis, most likely caused the observed reduction in urinary pH during week two of drug treatment.

Gross Pathology: At scheduled necropsy at the end of the drug treatment period, thymic involution and/or a reduction in thymus size was seen in the groups dosed with interferon (2/3 ♀), ribavirin (1/3 ♀), and the low (1/3 ♀)

and high-dose combination drug treatment groups (2/2 ♂ and 2/2 ♀). Thymic weights were lower in 1/3 low-dose ♂ (No. 33M), 1/2 high-dose ♂ (No. 23M) and 1/2 high-dose ♀ (No. 28F). The lower thymus weights correlated with thymic atrophy seen histopathologically.

Liver weights were increased in the groups administered interferon (2/3 ♀) and low-dose combination (2/3 ♀). These findings were considered spurious for the following reasons: (1) increased liver weights were not seen in the high-dose combination group, (2) there were no histopathologic findings in the liver which would have accounted for the increased liver weights, and (3) increased liver weights were not seen in a previous toxicity study in which interferon was administered to cynomolgus monkeys by subcutaneous injection at a dose of 3105 µg/m² every other day for one month.

Pale bone marrow was observed in 1/2 ♂ (No. 25M) and 1/2 ♀ (No. 29F) in the high-dose combination group at the conclusion of treatment.

All other gross and/or organ weight effects observed at the conclusion of the drug treatment interval appeared randomly distributed among the study groups and unlikely to be related to drug treatment. There were no test article-related necropsy or organ weight findings at necropsy at the conclusion of the off-dose recovery period.

Microscopic Pathology: Erythroid hypoplasia and mild-severe increases in myeloid to erythroid ratios (M:E) were seen in 3/3 high-dose combination ♂ at the conclusion of dosing. The bone marrow findings in the ♂ monkeys in the high-dose combination group were consistent with the non-responsive anemia (as discussed in the hematology section above). In contrast, ♀ monkeys in the high dose combination group at Week 5 generally had appropriately distributed marrow elements. At the end of dosing, one ♂ monkey in the low dose combination group (No. 33M) had erythroid hypoplasia with mildly increased M:E ratio while the remaining two monkeys had a mild left shift of erythroid elements. A single high-dose combination group ♀ was sacrificed on Day 32 with mild hypocellularity of the marrow section. Further, one ♀ monkey in the low dose combination group and one ♀ administered interferon alone (No. 15F) showed erythroid hypoplasia and mildly increased M:E ratio at the end of treatment. At the conclusion of the off-dose recovery period, the bone marrow progenitors of the two high-dose recovery group males were approaching control values, although mild erythroid hypoplasia was still evident in one animal (No. 36M).

Comments: 1) In general, the most profound (magnitude of effect and/or the numbers of animals effected) marrow changes were observed in the high and low dose combination treatment groups at the conclusion of dosing. Post-treatment recovery of marrow cellularity was evident, although residual tissue injury was apparent 5 weeks following drug cessation.

2) The observed histopathologic changes noted in the bone marrow of the drug treated animals closely relates to the changes noted for the peripheral blood.

Occasional mild-severe microcytosis was observed during treatment weeks 3-4 and during week 5 in 4/5 ♀ monkeys in the high-dose combination treatment group. Minimal-mild microcytosis occurred in 1/5 high-dose combination ♂ in week 4 and 5/5 during Week 5 of treatment. A low incidence (one to a few animals) of microcytosis was observed in the low dose combination treated ♂ and ♀ animals during Weeks 3-5 of dosing. Dose related minimal-mild hypochromasia, minimal poikilocytosis, and minimal-moderate anisocytosis were observed during treatment weeks 3, 4 and/or 5 among ♂ and ♀ animals in both combination drug treatment groups. Minimal-mild polychromasia was more consistent for ♀ monkeys than ♂ during Week 5 in either combination group. In addition, a dose related increase in the incidence of schistocytes and acanthocytes was observed in the combination treated animals beginning at week 4 (more prominent among the ♂ animals), and became more prominent during the post-treatment reticulocytosis.

Additional histopathologic effects noted at the conclusion of drug treatment included: 1) atrophy of lymphoid organs in animals administered interferon or interferon with ribavirin (higher severity generally in the combination drug groups), 2) increased iron deposits in the liver and spleen in all treatment groups, but with higher incidence/severity in the combination drug groups, 3) depletion of adrenal cortical lipid content in the interferon and combination treatment groups, and 4) minimal-moderate perivascularitis and injection site inflammation in animals dosed with interferon (alone or in combination with ribavirin).

- Comments:
- 1) The prominent anemia, along with the presence of an increased amount of iron pigment in liver and spleen and no increase in serum bilirubin, suggests an abnormal (increased) destruction of red blood cells in the periphery and/or extravascular spaces of the combination drug treated animals.
 - 2) The study results suggest that the combination of ribavirin and interferon has additive and/or synergistic effects in the induction of mild to severe anemia with/without the suppression of myeloid cells line. This effect may be due to bone marrow suppression by interferon and extravascular erythrocyte destruction by ribavirin.
 - 3) A relatively rapid erythrocytosis was evident following the cessation of combination drug treatment in both male and female animals.
 - 4) While the combination of interferon and ribavirin demonstrated additive and or synergistic effects on the production/induction of mild-severe anemia, there were no apparent previously unidentified toxicities associated with the administration of the combination drug product.

**APPEARS THIS WAY
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Appendix B: Carcinogenicity StudiesSummary of Carcinogenicity and Mutagenicity Study Findings:

The in vivo carcinogenicity studies and in vitro/in vivo mutagenicity studies performed with ribavirin were reviewed by the CDER CAC (Center for Drug Evaluation and Research - Carcinogenicity Assessment Committee) in executive session on 28 April 1998. The conclusions of this session are summarized as follows:

- 1) as regards the two oral gavage oncogenicity studies:
 - a) the studies were inadequately designed to assess the oncogenic potential of ribavirin, the drug doses utilized being too low as determined by the results of the oral gavage dose range finding studies, and
 - b) the studies were not conducted in accordance with the study protocols or were incomplete, and were not adequately reported /documented.
- 2) as regards the carcinogenic potential of ribavirin when administered to rats by dietary admixture:
 - a) the submitted study reports are sufficiently unclear as to be able to determine whether a single study, or multiple independent studies, were performed with ribavirin (see also NDA 18-859
 - b) multiple transfers of the study animals between research centers were performed while the study was in progress,
 - c) group housing of test animals was used during transfer(s) thus allowing for misidentification of the study animals,
 - d) there were inconsistencies in the reporting of the experimental design (i.e., number of animals per test group, animal numbering [identification] system, and test drug dose),
 - e) there were irreconcilable differences in the mortality and survivorship data (i.e. there were too many animals alive at the conclusion of the study based on the initial group sizes and the reported mortality for each treatment group),
 - f) there were no body weight, clinical chemistry and hematology, or survivorship data available for review from the on-study interval between 6 and 15 months, and
 - g) all histopathology data from animals which died, were euthanized moribund, or sacrificed for interim analyses during the interval from 6-15 months were lost from the study and were not available for interpretation of the experimental results.
- 3) as regards the mutagenic effects of ribavirin:
 - a) ribavirin increased the incidence of cell transformations and mutations in mouse Balb/c 3T3 (fibroblasts) and L5178Y (lymphoma) cells at concentrations of 0.015 and 0.03-5.0 mg/ml, respectively (without metabolic activation),
 - b) increased mutation rates (3-4x) were observed at concentrations between 3.75-10.0 mg/ml in L5178Y cells in vitro with the addition of a rat liver S-9 metabolic activation fraction,
 - c) when tested in the in vivo mouse micronucleus assay, ribavirin was clastogenic at doses of 20-200 mg/kg (IV; estimated human dose equivalent of 1.67-16.7 mg/kg, based on body surface area adjustment for a 60 kg adult), and
 - d) ribavirin was not mutagenic in a dominant lethal assay in rats at doses between 50-200 mg/kg when administered for 5 days (estimated human dose equivalent of 7.14-28.6 mg/kg, based on body surface area adjustment).

In summary, it was the conclusion of the committee that the results of the 2

oral gavage oncogenicity studies in the mouse and rat (18-24 months; doses of 20-75 and 10-40 mg/kg/day, respectively [estimated human equivalent doses of 1.67-6.25 and 1.43-5.71 mg/kg/day, based on body surface area adjustment for the adult; maximal animal drug exposure being approximately 1.3x and 0.2x the human systemic exposure to ribavirin based on 24 hour AUC at the recommended clinical dose of 1.2 grams per day]) are inadequate to draw conclusions as to the carcinogenic potential of ribavirin. The dietary admixture study results submitted were also deemed inadequate to support the safety assessment of ribavirin as regards carcinogenic potential. However, the results of all of the studies suggested that chronic ribavirin exposure might be related to an increased incidence of vascular lesions (microscopic hemorrhages in mice) and retinal degeneration (in rats). Ribavirin increased the mutation rate or caused chromosomal damage when tested in multiple in vitro and in vivo assays for genotoxic effects.

It was the conclusion of the committee, based on the positive genotoxic effects seen with ribavirin in multiple assay systems, the lack of adequate in vivo rodent carcinogenicity data and the extended period of human drug exposure (treatment regimen of 6 months duration), that: a) the product label for ribavirin should indicate that it may be a potential carcinogen (see the wording of the Carcinogenesis and Mutagenesis section of the proposed product label as contained in Appendix F of this document), and b) the sponsor, as part of a Phase 4 Post-Marketing Agreement, should be required to perform additional in vivo animal studies to assess the carcinogenic potential of ribavirin (see Review Summary and Recommendations).

**APPEARS THIS WAY
ON ORIGINAL**

Toxicity Studies Summary:

- 1) Eighteen Month Oral Gavage Oncogenicity Study In Mice With Ribavirin (No. 86-3068,
- 2) A Two Year Oral Gavage Chronic Toxicity/Oncogenicity Study In Rats With Ribavirin (No. 86-3066,
- 3) Two-Year Chronic Oral Toxicity Study With ICN 1229 (Virazole) In Albino Rats (March 11, 1974, Study No. 622-02600)

Toxicity Study Reviews:

- 1) Eighteen Month Oral Gavage Oncogenicity Study In Mice With Ribavirin (project no. 86-3068,

Six hundred CD-1 mice (300 male and 300 female mice; Charles River Breeding Lab., Kingston, NY) were randomly assigned to 1 of 5 treatment groups (60 animals/sex/group), using a standard design of 2 control groups and 3 treatment groups. Animals were housed individually in stainless steel wire mesh cages with ad libitum access to rodent chow and tap water. Actual environmental conditions in the housing space were as follows: temperature range 63-78°F (17.2-25.6°C), relative humidity range 6-86%, and lighting cycle of 12/12 hours. Mice were administered ribavirin (Lot # 03300786, at doses of 20 (group III), 40 (group IV), and 75 mg/kg (group V), or distilled water vehicle (groups I and II). Drug or vehicle were administered orally (in a final volume of 15 ml/kg) to all animals (includes both controls) by gastric intubation once/day for 18 months, beginning when the animals were six weeks of age. Observations for morbidity and/or mortality were performed twice daily, with detailed physical examinations performed 1/week. Body weight and food consumption were measured weekly between weeks -1 (pretest) and 13, and monthly thereafter. A satellite group of 10 male and 10 female animals were used for the assessment of baseline hematologic parameters prior to the initiation of drug dosing. Hematologic and clinical chemistry analyses were subsequently performed on 10 animals/sex/dose at 1, 3, 6, 12 and 18 months of treatment. Additional pooled plasma samples were taken from groups of 5 animals/sex/dose for measurement of plasma drug levels (results of these procedures were not provided in this report).

All surviving animals were sacrificed by exsanguination following 18 months of drug dosing. Histologic examinations were performed only on animals from control group 1 and those from the highest dose group (75 mg/kg/day). Histologic examinations were not performed on animals from control group 2 or the remaining dosed groups (unless the animals died before the end of the study (unscheduled death) or had lesions visible during a gross necropsy).

RESULTS

Plasma Drug Levels: Blood samples were taken on day 1 of dosing and at 3, 6, 12 and 18 months of treatment for the analysis of plasma drug levels. No information regarding plasma drug levels (C_{max}) or systemic exposure (AUC) was provided in this report. Thus, it is not possible to relate any of the observed toxicities as described below to measured differences in drug exposure.

Mortality: Males dosed at 20 or 40 mg/kg/day showed significantly ($p < 0.05$) higher mortality than either control or high dose males. Deaths in the two female control groups were significantly different, such that there were no differences in survivorship of female animals when compared with the combined control groups. (No explanation or historical data was provided which might

allow for the determination of which control group was atypical or unusual.) However, if comparisons were made with the control group showing fewer deaths, then there was a statistically significant increase in the incidence of premature death among females in the 75 mg/kg/day group. A table of survivorship is provided below.

Group Dose (mg/kg)	Survivorship				
	I 0	II 0	III 20	IV 40	V 75
Male					
Group Size	60 ^a	60	60	60	60
Survivors ^b	37	33	24	23	10
Data Rec. ^c	39	34	26	26	31
Chron. Table ^d	37	33	24	23	30
Female					
Group Size	60	60	60	60	60
Survivors ^b	22	37	31	35	27
Data Rec. ^c	26	37	32	36	27
Chron. Table ^d	22	36	31	35	26

^a Data records for animals no. 1002 and 1031 were not included in some tables and the animals were reported as missing.

^b Survivorship as computed from the mortality/sacrifice data on pg 8 of Appendix H (Vol 1).

^c Survivorship as determined from the data records of body weight and food consumption during week '78' of the experiment (pages 918-938 of Appendix D).

^d Survivorship as determined from the chronological listing of deaths as contained on pages 2-15 of Table V, Appendix H.

A large discrepancy in the number of male survivors in the 75 mg/kg/day group was noted when comparing the data from the mortality/sacrifice table on pg 8 of Appendix H (Vol 1) and the chronological listing of deaths as contained on pages 2-15 of Table V, Appendix H (Vol 4). No explanation for this discrepancy is evident other than poor record keeping and quality control.

According to the information presented in the table above a total of 17 animals died spontaneously during the approximately 8 days during which terminal necropsies were being performed (determined by subtracting the number of animals terminally sacrificed^a from the number of animals alive on the final day of data [body weight and food consumption] collection^b). The number of deaths reflects a nearly 5x increase in the incidence of spontaneous mortality than was observed during any of the preceding 3 observation periods (i.e., 3 observation periods of 4 weeks duration each). There is no explanation provided for the increase in spontaneous deaths.

Physical Signs: The incidence of abnormal signs (i.e., poor grooming, skin lesions, abnormal posture and movement, etc.) was comparable for the control and drug treated animals. However, comments in the pathology report suggest a slightly increased incidence of cutaneous ano-genital ulcers and alopecia in drug treated versus control animals.

Body Weight and Food Consumption: Ribavirin administration resulted in a dose related lowering of absolute body weight and weight gain in males and females early in the study (1-4 months) which was maintained for males throughout the study. These effects were generally statistically significant among males and females in the 75 mg/kg/day treatment groups (Male: mean final body weight was decreased by <10% while mean weight gain was decreased by 23%; Female: mean final body weight was decreased by <5% while mean weight gain was decreased by approximately 15%), and were sporadically significant for other dose groups. The reductions in body weight (<10% over the duration of the study) observed in this study are not considered of biological significance or as evidence of drug delivery.

Both increases and decreases in food consumption (significant at $p < 0.05$) were noted among treated animals (male and female) during the course of the study.

However, there were no consistent patterns evident in these differences.

Hematology: Erythrocyte count (RBC), hemoglobin (HGB) and hematocrit (HCT) were decreased among male and female animals treated with ribavirin at 75 mg/kg/day. Platelet (PLT) counts were increased in high dose males at termination and in high dose females at 6 months. Sporadic differences in RBC, HGB and HCT were noted during the study for animals in the 40 mg/kg/day treatment group. Hematologic parameters for male and female animals in the high dose treatment groups are given in the table below.

Hematologic Changes at 1 and 18 Months (75 mg/kg/day)				
	RBC (mil/ μ l)	HGB (g/dl)	HCT (%)	PLT ($10^5/\mu$ l)
Male - 1 Month				
Control	8.26	14.6	42	12.45
75 mg/kg	7.81	13.4	40	14.05
- 18 Months				
Control	7.67	13.9	39	16.97
75 mg/kg	6.09	11.3	32	26.67
Female - 1 Month				
Control	8.20	14.9	44	10.87
75 mg/kg	7.82	13.8	41	12.20
- 18 Months				
Control	7.64	13.6	38	14.55
75 mg/kg	6.42	11.9	34	17.86

Organ and Organ/Body Weight Ratios at Termination: Absolute and relative weight of the spleen was significantly increased (versus control) for male and female animals in the high dose (75 mg/kg/day) group. Histological examination of the tissues suggested that this effect was due to an increase in extramedullary hematopoiesis. Other differences seen at termination included decreased testicular weight (significant decrease in absolute weight) among males in the middle (40 mg/kg/day) and high (75 mg/kg/day) dose groups, and a slight decrease in ovarian weight among the high dose females. Evidence of hypospermatogenesis was noted during histologic evaluation of the testicular tissues of male animals from the high and middle dose groups.

Histologic examination: The incidence of selected lesions in mice administered ribavirin for 18 months are presented in the following table.

Summary Table of Histologic Abnormalities Observed in
Mice Treated With Ribavirin For 18 Months

Lesion	Dose (mg/kg)				
	0	0	20	40	75
Brain - abscess or focal hemorrhage					
male	0/58	0/60	1/60	2/59	0/60
female	1/56	0/60	1/60	3/60	1/60
Dermatitis -					
male - ear	8/58	7/60	10/60	11/59	17/60
female - ear	11/56	3/60	10/60	13/60	14/60
Epididymis -					
hypospermia	28/58	36/60	31/60	37/60	49/60
abscess	0/58	0/60	0/60	0/60	1/60
Esophagitis - chronic, nonsuppurative					
male	4/58	1/58	1/59	2/60	6/60
female	1/56	1/60	3/59	1/60	6/60

Summary Table of Histologic Abnormalities Observed in
Mice Treated With Ribavirin For 18 Months (continued)

Lesion	Dose (mg/kg)				
	0	0	20	40	75
Heart - abscess					
male	0/58	0/60	1/60	2/60	0/60
female	0/57	0/60	1/58	3/60	2/60
Intestinal Tract (large, cecum)					
edema, submucosal					
male	0/58	0/60	0/60	6/58	0/59
female	0/57	3/60	1/59	0/59	1/60
<u>nematodiasis</u>					
male	6/58	11/60	11/60	16/58	5/60
female	12/57	12/60	9/59	10/59	4/60
Ovary -					
<u>Granulosa cell tumor</u>					
0/57		0/60	1/60	0/59	0/59
<u>Granulosa-theca cell tumor</u>					
0/57		0/60	0/60	1/59	1/59
Seminal Vesicle - acute or subacute vesiculitis					
0/57		2/59	2/59	3/60	5/60
Skin - surface ulceration					
male	2/58	3/60	6/60	6/60	5/60
female	0/56	0/60	1/60	4/60	1/60
Spinal cord - abscess/focal hemorrhage of cord/meninges					
male	0/58	0/60	0/60	1/60	1/59
female	0/56	0/60	1/60	2/60	0/60
Spleen - <u>histiocytic sarcoma</u>					
male	0/58	0/59	0/59	0/60	0/60
female	0/57	0/60	0/60	0/60	2/60
Stomach - mucosal erosion					
male	1/58	0/60	0/60	4/59	2/60
female	1/57	1/60	0/60	3/60	0/60
Testis - hypospermatogenesis					
30/58		35/60	36/60	44/60	49/60
Thymus - abscess					
male	0/56	0/59	1/59	1/58	1/60
female	0/57	0/60	2/59	1/60	1/60
Thyroid - thyroiditis, nonsuppurative					
male	0/58	0/56	0/57	1/60	0/57
female	0/56	0/60	2/56	1/57	5/60
Uterus - endometritis					
1/57		0/60	0/60	0/60	4/60
Vagina - vaginitis					
0/57		0/58	0/60	0/55	2/57

The histologic findings suggest that ribavirin administration increased the incidence of testicular and epididymal hypospermia (at doses of 40 and 75 mg/kg/day), and induced a recurrent pattern of abscesses and/or focal hemorrhages in the heart, brain, spinal cord and epididymides of the drug treated animals (all doses).

Comments:

- 1) The protocol indicates that blood samples were taken on day 1 of dosing and at 3, 6, 12 and 18 months of treatment for the analysis of plasma drug levels. The data/results were not reported in the submission.

2) The basis for ribavirin dose selection was not given. However, on the basis of a 13-week dose range-finding study conducted by Bio/dynamics Inc. (using the same route of administration and the same strain of mouse), the doses selected for evaluation appear to have been too low. Based on the previous study, the expected high dose for this study should have been approximately 150 mg/kg/day. The high dose used in the present study (75 mg/kg/day) is potentially inadequate for testing the oncogenic capacity of ribavirin.

3) Comments contained in the pathology report indicate that there was a slight increase in the incidence of alopecia and cutaneous anogenital ulcers in drug treated animals versus controls. Further, histologic evaluation of selected tissues suggested an increased incidence of gastric mucosal erosion and intestinal irritation among the drug treated animals. These findings are consistent with an anti-proliferative effect of ribavirin and a potential local irritant effect.

4) The histology data suggest that ribavirin administration at doses of 40 and 75 mg/kg/day only slightly increased the incidence of testicular and epididymal hypospermia observed following 18 months of drug administration. However, it should be noted that similar findings were previously seen when CD-1 male mice were dosed for 1-3 months duration. Further, in the earlier study the difference between drug treated and control animals was much more apparent as the incidence of hypospermia among the control animals (age 4-6 months) was very low. Because hypospermia is normally observed in aged animals of the CD-1 strain it is likely that the control animals 'caught-up' with the drug treated animals by the termination of the study, thus masking the earlier effect. The effects of ribavirin induced testicular toxicity on general reproductive performance and teratogenicity have not been determined.

5) Neoplastic changes, including ovarian granulosa-theca cell tumors (intermediate and high dose) and histiocytic sarcoma of the spleen (high dose), were evident only in female animals treated with ribavirin for 18 months. Although several tumors were noted, it remains that the drug doses used in the study were potentially inadequate for testing the oncogenic capacity of ribavirin.

6) Oral treatment regimens for humans have varied between 600 and 1800 mg per day (i.e., 10-30 mg/kg/day for a 60 kg adult) (Goodman and Gilman's (ed), The Pharmacologic Basis of Therapeutics, p. 1193, 1990). On a dose per body surface area basis, the doses used for mice in the current study are lower than those given to humans (human equivalent doses of 1.7-6.25 mg/kg, based on body surface area adjustment). The development of pharmacokinetic data for the mouse would help in the comparison of mouse and human exposure and risk estimates for human exposure to ribavirin.

7) Although the incidence in individual tissues is low, there appears to be a recurrent pattern of abscesses, focal hemorrhages or abnormal proliferative responses in the heart, brain, spinal cord, epididymides, spleen, thyroid and thymus tissues of the drug treated animals. Abscesses were not identified as sterile or due to an infectious agent. Because sterile abscesses may be associated with necrotic or ischemic tissues resulting from vascular abnormalities (including hemorrhage), the data for these two lesions have been combined. It is unlikely that the gastrointestinal nematodiasis noted in the test animals could account for the increase in lesions within the CNS and spinal cord.

The increased incidence of focal hemorrhages and abscesses had not been

previously identified. Abnormal proliferative responses have been previously noted in the bone marrow and mucosa of animals receiving chronic ribavirin treatment (1-3 months). Due to the serious nature of internal hemorrhage this effect warrants further evaluation, particularly as to whether the incidence of lesions increased with longer duration drug exposure.

8) A significant incidence of nematodiasis was noted in all of the test groups. This finding significantly hinders the determination of a causative relationship between drug treatment and pathological outcomes; this is particularly true as regards the incidence of gastrointestinal, liver and gastric lesions. The use of 'dirty' animals in the procedure places the validity of the entire study and its results in question.

9) There are no explanations given for the inconsistencies in the mortality/survivorship data.

10) A total of 17 animals died spontaneously during the approximately 8 days during which terminal necropsies were being performed. This number of deaths reflects an approx. 5x increased incidence of mortality than that which was observed during the preceding 3 observation periods (i.e., 3 observation periods of 4 weeks duration each). There is no explanation provided for the increase in spontaneous deaths.

2) A Two Year Oral Gavage Chronic Toxicity/Oncogenicity Study In Rats With Ribavirin (project no. 86-3066,

Groups of 60 6-week-old Charles River CD rats of each sex were given 0, 0, 10, 20, or 40 mg/kg ribavirin in distilled water by gavage, once per day for 104 weeks (all animals including both control groups were dosed on a daily schedule). Satellite groups of 10 animals/sex/dose group were killed at 12 months for an interim evaluation. Hematologic and clinical chemistry analyses were conducted on 10 animals/sex/dose group at various times during the study and on 20 animals/sex/dose group at month 12. Histologic examinations were performed only on animals from control group 1 and the highest dose group. Histologic examinations were not performed on control group 2 or on the other dosed groups unless the animals died before the end of the study (unscheduled) or had gross lesions.

RESULTS

Mortality: Survival of males from the drug treated groups (55%-57%) was greater than that of male controls (30%). Survival of females from the dosed groups (53% for the 10 and 20 mg/kg groups and 38% for the 40 mg/kg group) was greater than that of female controls (32%).

Body Weight and Food Consumption: Mean body weights of the 40 mg/kg/day group of male rats were about 12% lower than those of controls until week 76-80; thereafter body weights were not dose related or were comparable to those of controls. The mean body weight of the 40 mg/kg/day group of female rats was more than 10% lower than those of controls (up to 16% lower) throughout most of the study. For the satellite group of rats killed at 12 months, the mean body weight of males in the 20 mg/kg group was lower than that in the 40 mg/kg group and was significantly lower (>10% lower) than that of the controls. Food consumption by dosed and control groups was comparable throughout the experiment.

Hematology: HGB and HCT were slightly (significant) lower than those of controls (decrease of < 10% vs. control) through month 12 of the study for males in the 40 mg/kg/day group, and throughout the study for females in the

same dose group. At some sampling times, values for other dosed groups were also slightly lower than the controls. Platelet counts were significantly increased (up to approximately 25%) at most time points up to 18 months for males and females treated with ribavirin at 40 mg/kg/day. Erythrocyte counts were not consistently or significantly affected. Mean corpuscular volume was not effected by ribavirin treatment at any dose tested.

Previously reported data suggests that decreases in HGB and HCT which are frequently associated with ribavirin administration are due to suppression of the bone marrow and a direct extravascular hemolytic effect. In the present study the decreases in hematologic parameters were quite modest (<10%) and of questionable biologic significance as there was no compensatory increase in mean corpuscular volume and reticulocyte count.

Clinical Chemistry: There were no drug-related effects on clinical chemistry parameters observed in the study.

Plasma Drug Level Assessment: The sponsor separately submitted the results of a plasma drug level study (assays performed 4-5 years after the acquisition of the samples) which was conducted for dose validation in the two year rat oncogenicity study with ribavirin. The results of the plasma drug levels measurements are outlined in the following table.

Dose mg/kg/day		N=	Blood Drug Levels (µMoles/L) in the SD Rat						
			Test Day 1	30	90	180	360	540	720
10	♂	13	1.3	0.8	1.1	1.0	0.5	0.6	1.2
	♀	12	1.2	1.6	0.6	0.7	0.3	0.8	0.7
20	♂	9	3.0	2.3	2.8	2.8	0.8	1.7	1.7
	♀	12	3.4	1.5	1.3	0.8	0.3	1.1	2.5
30	♂	13	4.4	3.3	0.4	3.1	0.7	1.6	3.1
	♀	9	6.2	7.9	4.0	4.0	0.4	1.7	2.9

Comments: 1) Whole blood samples were drawn on drug dosing days 1, 30, 90, 180, 360 and 720 of the study. Only on day 540 was the blood sample separated to achieve a cell free plasma fraction. All samples were frozen and retained for assay following the completion of the in-life phase of the study. Because the cells contained in the whole blood samples lysed during the freeze-thaw procedure, the resultant assayed drug levels may not accurately reflect actual plasma drug concentrations (due to the release of previously intracellular drug [or drug metabolite] pools, or by dilution of the plasma through the addition of the intracellular fluid volume).

2) As noted above, all samples were frozen and retained for assay following the completion of the in-life phase of the study (in actuality, samples were not assayed until 2-3 years after the completion of the study [4-5 years from the acquisition of the days 1, 30 and 90 samples]). There are no data available regarding the stability and/or degradation of ribavirin when stored frozen in blood or plasma. Therefore, levels presented in the preceding table should not be considered as necessarily an accurate quantitative representation of the test animals drug exposure, but should instead be considered only as a qualitative representation of relative drug exposure between different test groups.

3) Numerous samples from nearly all of the study animals in all three dose groups were reported as containing ribavirin in concentrations below the limit of detection (0.05 µM/L) for the RIA procedure. These 'missing' values have been assigned a value of zero for the computation of the drug levels presented above.

4) The results presented in the preceding table suggest only that the drug treated animals were subjected to some systemic exposure to ribavirin, and the level of the systemic drug exposure increased in conjunction with increases in the administered drug dose.

Histologic examination: The incidences of selected lesions in rats administered ribavirin for two years are presented in the following table.

Lesion	Dose (mg/kg)				
	0	0	10	20	40
Adrenals (Cortical)					
Carcinoma					
female	0/55	0/56	0/58	2/59	2/54
Cysts					
female	2/55	0/56	0/58	2/59	8/54
Bone marrow					
hypercellularity					
male					
12 mo	3/13	2/18	7/14	9/16	12/16
2 yr	20/56	21/51	28/55	24/53	34/52
female					
12 mo	1/13	7/14	5/11	4/11	2/16
2 year	29/55	20/56	25/57	35/58	22/53
Liver - Bile Duct Hyperplasia					
male					
12 mo	2/13	2/19	2/15	5/16	5/16
2 yr	36/57	25/51	38/55	42/54	48/54
female					
12 mo	4/15	1/14	1/11	4/11	3/16
2 yr	13/55	22/56	31/59	31/59	17/54
Ovary					
Cysts	5/53	2/56	10/59	11/59	10/54
Hyperplasia	3/53	1/56	1/59	3/59	7/54
Retinal atrophy					
or degeneration					
male	2/57	5/51	11/55	13/54	21/53
female	11/54	14/56	20/59	30/59	28/54
Thyroid C-cell Adenoma or Carcinoma					
female	7/54	7/54	14/58	9/58	13/54

Comments:

1) The basis for ribavirin dose selection was not given. However, on the basis of a 13-week dose range-finding study conducted by (using the same route of administration and the same strain of rat), the doses selected for evaluation in the present study appear to be too low. Based on the previous study, the expected high dose for this study should have been approximately 80 mg/kg/day. The high dose used in the present study (40 mg/kg/day) is inadequate for testing the oncogenic capacity of ribavirin.

2) Ribavirin administration clearly resulted in retinal atrophy/degeneration in both male and female rats. While retinal degeneration is common with increased age in the albino laboratory rat, ribavirin administration appears to have significantly enhanced the frequency and degree of the lesions. The significance of this observation to the human condition is not known, although extreme caution and close observation of retinal conditions are recommended when ribavirin is administered chronically or to neonates/ pediatric patients.

3) Administration of ribavirin was associated with hyperplastic responses of the bone marrow (male and female animals), bile ducts (male and female animals), and ovarian epithelium. These effects were evident following 12 months of drug administration, although the effects were generally more prominent at the conclusion of the 2 year dosing interval. This effect is likely a compensatory response to the non-proliferative effects of ribavirin previously noted in short-term and acute high dose studies.

4) Increased incidences of non-neoplastic cysts were observed in the adrenal cortical region (high dose) and ovaries (all doses) of female rats. Neoplastic changes, including thyroid C-cell (low and high doses) adenoma and carcinoma, and adrenal cortical carcinoma (intermediate and high dose), were evident only in female animals treated with ribavirin for 2 years.

5) Oral treatment regimens for humans have varied between 600 and 800 mg/kg per day (Goodman and Gilman's (ed), The Pharmacologic Basis of Therapeutics, p. 1193, 1990). On a dose per body surface area basis, the doses used for rats in the current study are lower than or barely equivalent to those given to humans. The development of pharmacokinetic data for the rat would help in the comparison of rat and human exposure and risk estimates for human exposure to ribavirin.

6) Ribavirin triphosphate (a possible candidate for some toxic effects, including extravascular hemolysis) accumulates in the erythrocytes of humans following repeat dosing (a plateau is generally achieved in 1-3 weeks of daily dosing) with an elimination half-life of approximately 40 days (Knight, V. et al. J. Infect. Dis. 158: 443-448, 1988). In humans then, the period of high exposure to and of potential toxicity from ribavirin is protracted (up to several months) even following a relatively short period of drug treatment (1-3 weeks). Thus, the long-term consequences of ribavirin administration must be defined so that they may be carefully considered in the assessment of therapeutic risk/benefit analyses.

7) Small decreases (<10%) in hemoglobin and hematocrit, and increases in platelet count were observed at similar doses in the previous 13-week dose finding study. The similarity of results suggest that for a particular dose, hematologic effects after 18 months of drug administration are no worse than (and potentially less than) after 3 months. In the present study the decreases in hematologic parameters were quite modest and of questionable biologic significance as there was no compensatory increase in mean corpuscular volume and reticulocyte count.

The study⁷ described below was NOT certified as having been conducted in accordance with the Good Laboratory Practice Regulations, Title 21, Part 58 (21 CFR Part 58) Dec. 22, 1978, as these regulations were not in effect at the time of the study.

3) Two-Year Chronic Oral Toxicity Study With ICN 1229 (Virazole) In Albino Rats (March 11, 1974, Study No. 622-02600)

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Groups of 70 Charles River albino rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) of each sex were given 0, 30, 60, or 120 mg/kg/day of ribavirin as a dietary admixture for 104 weeks. Ten animals/sex/dose were included in the design for an interim sacrifice at 6 months. Fresh diet was prepared weekly, with the amount of test material added to the diet being recalculated periodically to adjust for changes in the body weight and food consumption of the animals.

7 The study (or studies) reviewed in this section were originally submitted to NDAs 18-859 with documentation suggesting that a single study had been initiated, but that the study had been moved between test sites on two occasions. Subsequently, Schering corporation has re-evaluated and resubmitted portions of the study reports, suggesting that the data reflects portions of two independent studies initiated and conducted at separate test sites. However, regardless of the singularity or plurality of studies conducted, there is agreement between the Sponsor and Agency that the study conduct, results and documentation are inadequate to allow for valid conclusions.

Investigations by the Non-Clinical Laboratories Studies Branch, FDA, have determined that **many studies** performed by [redacted] had been conducted under circumstances that were fraudulent (this includes all drugs/compounds tested by [redacted]). The FDA has since required that **all studies**, regardless of the date of completion (or the drug), conducted by [redacted] must have been validated by a third party and the data must be available for FDA validation before the studies can be considered for safety review.

9 Within the body of the report there are references to the study site having been moved (approximately 15 months into the study) from a facility in _____.

However, documentation also suggests, although it does not outrightly state, that the study had been previously moved from the [redacted] to the [redacted]. This move apparently occurred sometime between the 6 month interim sacrifice (reported by [redacted] and the [redacted], move which occurred at 15 months.

Observations of morbidity and/or mortality were performed daily. Animals were weighed (all animals) and food consumption measured (10 animals/sex/group) once each week through week 13 of the study and monthly thereafter. Hematologic, clinical chemistry and urinalysis were performed on 10 animals/sex from the control and high dose groups (all groups if differences were found initially) following 3 and 6 months of drug administration. Ophthalmologic examinations were performed on 10 animals/sex from the control and high dose group at 3 months. Satellite groups of 10 animals/ sex/dose group were euthanized at 6 months for an interim evaluation. Histologic examinations were to be performed on all animals.

RESULTS

Months 0-6

Body Weight: Decreased weight gain and reduced body weight (statistically significant reductions) were evident among dosed females within one week of the start of drug dosing. After six months of drug administration, the mean body weight of female animals from the treatment groups was reduced in a dose related manner (96%, 89% and 83% of control for the low, med. and high dose animals) versus that of the controls. Among male animals, body weight was significantly reduced only in the medium (92.5% of control) and high dose groups (80.0% of control). Decreased body weight and reduced weight gain was evident among male animals beginning two weeks following the initiation of drug administration.

Food Consumption: Mean food consumption was not consistently effected among male animals at any test dose (all group mean were within 5% of food consumption by the control group). However, among female animals, food consumption was reduced in a drug dose related manner at three (92.5%, 87.4% and 85.1% for the low, med. and high dose) and 6 months (93.4%, 88.2% and 86.7% for the low, med. and high dose) of treatment. Food utilization (i.e., weight gain per grams of food consumption) was generally reduced among male and females from all drug treatment groups versus the controls.

Morbidity and Mortality: There were no physical signs reported during the first six months of drug treatment. Mortality was slightly increased among males in the 120 mg/kg/day treatment group (Male: control= 0/60, 30 mg/kg= 2/60, 60 mg/kg= 1/60, 120 mg/kg= 8/60; Female: control= 3/60, 30 mg/kg= 0/60, 60 mg/kg= 1/60, 120 mg/kg= 3/60).

Hematology, Chemistry and Urinalysis:¹⁰ Hemoglobin, hematocrit and erythrocyte counts were decreased among male and female animals in the high dose group (120 mg/kg/day) following 3 and 6 months of drug administration. While, mean corpuscular volume and reticulocyte counts were increased by exposure to ribavirin, particularly among animals in the 120 mg/kg/day treatment group. See the following table for a summary of the hematologic effects. Mean corpuscular hemoglobin and hemoglobin concentration, differential leukocyte

¹⁰

Note: The animals identification numbers used in the hematology, clinical chemistry and urinalysis data tables do **NOT** correspond with the numbering system used in any of the other data tables contained in this report. Failure on the part of the sponsor to use consistent identification of samples or to include a table of cross-indices means that it is not possible to identify hematologic or other parameters with individual animals.

counts and prothrombin time were all unaffected by treatment with ribavirin at any test dose.

All clinical chemistry and urinalysis values were comparable among drug treated and control animals at 3 and 6 months, except that the serum alkaline phosphatase activity of animals in the high dose condition was approximately 1/2 that of the controls.

Hematologic Parameters Following 6 Months of Dosing						
Nominal Dose		HGB g/dl	HCT %	RBC 10^6mm^{-3}	MCV μ^3	Retic #/10 ³ RBC
Male	0 mg/kg	15.7	40.5	8.36	49	13
	30 mg/kg	14.3	36.7	7.75	47	19
	60 mg/kg	13.9	35.7	7.49	47	26
	120 mg/kg	12.6	33.7	5.71	60	24
Fem.	0 mg/kg	15.7	39.5	7.56	53	9
	30 mg/kg	13.9	35.7	7.49	47	27
	60 mg/kg	14.3	36.1	7.39	49	27
	120 mg/kg	11.0	29.6	5.71	64	30

Ophthalmologic Examination: Moderate to marked bilateral loss of retinal and choroidal blood perfusion with a resultant gray-white fundic appearance was noted in three female animals from the high dose group. Examinations were performed on only 10 animals/sex from the high dose and control groups.

Gross and Histologic Pathology: An increased incidence of alopecia was noted among animals of the high dose group as compared with that of control animals. An increase in the absolute (male and female) and relative heart to brain weight ratio¹¹ (female only) was evident among animals in the 120 mg/kg/day group. Absolute (decrease) and relative (increase vs. brain) weight of the liver was effected in males and females from the high dose group and females from the medium dose condition. Males in the 60 mg/kg/day treatment condition displayed a decrease in absolute liver weight only. Additionally, absolute kidney weight was decreased among high dose males, while relative (to brain) weight of the adrenals was increased in females of the same group. No other gross lesions or differences in organ weight were noted in the animals sacrificed or found dead during the first 6 months of the investigation.

Histologic evaluation of tissues from the euthanized animals revealed an increased incidence and severity of hypercellularity (mast cells) of the bone marrow in high dose animals versus the controls. Further, in two females from the 120 mg/kg/day group there was evidence of focal acanthosis and hyperkeratosis of the epidermis in areas affected by alopecia.

RESULTS

¹¹ Relative organ weights were computed versus body weight and brain weight. Because, ribavirin admin. had induced a significant decrease in weight gain, nearly all organ to body weight ratios were different for treated and control animals. The presented data utilizes comparisons with brain weight (a more conserved organ) for the purpose of clarity.

Months 7-15

There are no data or summary reports of the events or conduct of the experiment during the in-study dosing period of 7-15 months.

RESULTS**Months 16-24**

Transfer of Animals: Immediately prior to transfer, a total of 8 animals/sex and dose group were euthanized for an interim analysis. All remaining animals on study were transferred from _____ during the 66th week of drug administration. The drug groups and number of residual animals transferred to the HRC are as specified in the table below.

Nominal Dose mg/kg/day	Number of Animals Received by Huntingdon Res.			
	0	30	60	120
Male	65	44	44	39
Female	60	49	47	45

Comment: According to the description of this study as documented by _____ there were originally 70 animals of each sex assigned to each dose group. It should be noted that the animal identification system (numbering) corresponds to 70 animals/sex/dose. As previously discussed, 10 animals/sex/dose were sacrificed at 6 months on-study and an additional 8 animals/sex/dose were sacrificed at 15 months on-study (just prior to transfer). Assuming that there were NO spontaneous deaths, then the maximum number of animals of either sex remaining in any group should be 62. If spontaneous deaths as reported for the 0-6 month period (no mortality data are available for months 7-15) are also subtracted from the original group size, then the numbers of male and female animals remaining in the control condition are impossible to account for (i.e., there are too many live animals).

Drug Intake From Diet: The mean drug intake of male and female animals from each of the drug groups is contained in the following table and is presented by test site.

Nominal Dose mg/kg/day	Dietary Intake of Ribavirin			
	0	30	60	120
Male	0	16	33	70
Female	0	22	43	95
Male	0	16	33	64
Female	0	22	43	99

* The time period used for the computation of dietary drug intake and the numbers from which intake was derived, were not included in the report.

** Dietary intake of ribavirin was determined as the mean for months 16-24, inclusive.

Food Consumption: Average food consumption was not consistently effected among male or female animals in any test group versus the consumption by controls.

Body Weight: Group mean body weight was not consistently effected among male and female animals in any test group between months 16-24 versus the

corresponding controls. However at the time of terminal sacrifice, females from the high dose group weighed significantly less than control females.

Morbidity and Mortality: Except for an increased incidence of alopecia among ribavirin treated animals, there were no other physical signs reported during the 16-24 month treatment phase. The rate and incidence of mortality was comparable for all groups between months 15 and 21, while mortality increased among males and females from the control condition during the final 3 months of the study¹². At termination, survival of males from the high dose condition was lower than that of the controls, low and intermediate dose groups. Females in the control and high dose groups showed similar survival, while low and mid dose groups displayed a higher percent survival than control.

Hematology, Chemistry and Urinalysis: Hemoglobin, hematocrit and red blood cell counts were decreased among male rats from all ribavirin treated groups after 72 weeks of drug administration. Ribavirin treated female animals did not show differences from control. The effects in males were not dose related. Similar effects were noted among ribavirin treated animals at 104 weeks on-study (experiment termination). However, also noted among female rats was a dose related increase in neutrophil counts.

Blood chemistry analyses were performed at 65 and 104 weeks on-study and revealed decreased calcium (high dose females [65 weeks] and males [104 weeks]) and increased phosphorus (males and females from the mid and high dose groups; 65 weeks) in treated animals. Analyses revealed inconsistent variations in BUN (decrease; low and mid-dose males; 65 weeks), LDH (decrease; mid and high dose males; 65 weeks), glucose (decrease; low and high dose females; 65 weeks), albumin (decrease; high dose females; 65 weeks), and cholesterol (decrease; mid and high dose males; 104 weeks). Urinalysis which was performed at 93 and 102 weeks produced comparable values for drug treated and control animals, except that urine specific gravity was increased in a dose related manner.

Ophthalmologic Examination: Examinations were performed on all animals at 65 and 102 weeks on study. Focal retinal degeneration was noted in 31 animals but appeared to be randomly distributed among all groups.

Gross and Histologic Pathology: An increased incidence of alopecia and ulceration of the skin near the base of the tail was noted among male and female animals from the high dose group. At terminal sacrifice, the absolute weights of the liver and kidneys of drug treated animals (male) were statistically significantly less than for the controls. Both heart and adrenal weights were increased (statistically significant) among females from the high dose group. When expressed as a percent of body weight, there was a decrease in the relative weight of the liver in males and, an increase in the relative weight of the heart and adrenals of ribavirin treated females.

Histologic examination of tissues from the animals sacrificed after 15 months of ribavirin administration showed: a) an increased incidence of atrophy of the testes and seminiferous tubules of male rats from the mid- (3/8) and high (5/7) dose groups versus control (0/8), b) an increased incidence of mammary galactocoeles in females from all dosage groups (control - 0/8, low - 3/8, mid - 1/6, high - 5/7), c) a slight increase in the incidence of hepatocellular hyperplasia among high dose females (control - 3/8, high - 6/8) versus control, and d) a small increase in the incidence of uterine endometrial cysts

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No explanation for the increase in mortality among control animals was given by the sponsor or, was evident from the histology data.

(control - 1/8, low - 2/8, mid - 3/8, high - 3/7) and squamous metaplasia (control - 2/8, high - 3/7) among dosed females. There were no additional findings at the termination of the study.

The incidence of selected lesions is shown below.

Lesion	Dose (mg/kg)							
	0	30		60		120		
<hr/>								
	<u>Terminal Sacrifice</u>							
Mammary Gland	M	F	M	F	M	F	M	F
No. Examined	20	22	14	30	10	23	5	17
Fibroadenoma	1	3	0	7	0	4	0	4
Papillary Cyst adenocarcinoma	1	0	0	1	0	0	0	0
Papillary Cyst adenoma	0	4	0	2	0	5	0	5
Adenocarcinoma	0	1	0	5	0	0	0	2
Adenoma	0	0	0	1	0	3	0	2
Pituitary	M	F	M	F	M	F	M	F
No. Examined	29	24	22	28	26	30	17	18
Adenoma	10	13	8	18	12	20	5	14
% incidence	34	54	36	64	46	67	29	78
 <u>Spontaneous Deaths and Moribund Sacrifice</u>								
Mammary Gland	M	F	M	F	M	F	M	F
No. Examined	18	26	4	14	8	13	4	13
Fibroadenoma	0	10	0	4	2	7	0	1
Papillary Cyst adenocarcinoma	0	0	0	2	0	1	0	0
Papillary Cyst adenoma	0	7	0	4	0	3	0	0
Adenocarcinoma	0	4	0	0	0	0	0	2
Adenoma	0	0	0	0	0	0	0	2
Pituitary	M	F	M	F	M	F	M	F
No. Examined	17	23	7	13	7	8	13	13
Adenoma	8	17	4	9	3	6	5	5
% incidence	47	74	57	69	43	75	39	39
 <u>Cumulative Incidence</u>								
Dose (mg/kg)	0	30		60		120		
Pituitary	M	F	M	F	M	F	M	F
No. Examined	46	47	29	41	33	38	30	31
Adenoma	18	30	12	27	15	26	10	19
% incidence	39	63	41	66	45	68	33	61

* no histologic examination or unscheduled deaths only

The following table specifies the numbers of animals undergoing terminal necropsy, spontaneous death, or moribund euthanasia at the

Nominal Dose mg/kg/day	Number of Rats Examined			
	0	30	60	120
Terminal Necropsy				
Male	33	25	29	19
Female	25	33	33	23
Spontaneous Death and Moribund Euthanasia				
Male	31	18	14	19
Female	36	17	15	22

Comment: Adding the numbers of animals from the two categories listed above (summed within sex and dose group) should equal the number of animals transferred to the HRC from [redacted] Comparison of the group totals with those shown previously (see table under animals transferred), indicates that there is one male animal missing from each of the control, low and middle dose groups, and that there is one extra female animal in each of the control, low and middle dose groups. These inconsistencies are not accounted for.

The increased incidence of pituitary adenomas in animals sacrificed at the termination of the study suggest that ribavirin may have a slight carcinogenic potential. However, the findings obtained from animals found dead or sacrificed moribund (months 15-24) and the cumulative data for all animals do not confirm this trend. The data regarding the incidence of mammary adenomas are unclear, as the basis for categorization of lesions was not defined and the categories are not mutually exclusive (it is unclear whether a single lesion in one animal may appear under multiple categories, or whether a single animal with multiple different lesions will appear in only one category related to the primary lesion type). Individual histology reports were not included in the submission and therefore are unavailable for clarification of the ambiguity.

Comments: 1) This study was conducted in part by [redacted]. It has since been determined by the Non-Clinical Laboratories Studies Branch of FDA, that many studies performed by [redacted] were conducted under fraudulent circumstances. Thus any study conducted by [redacted] must have been validated by a third party and the data must be available for FDA inspection before the [redacted] study can be considered for determining the safety of a new drug.

Due to several inadequacies in the newly submitted rat and mouse oncogenicity studies (NDA 18-859, submissions 048 and 050), the new data are not considered to be acceptable non-confirmatory replacement studies for the [redacted]

2) This study predates the effective date of the Good Laboratory Practices regulations, Title 21, Part 58 (21 CFR Part 58) Dec. 22, 1978.

3) The conduct and reporting of this study does not meet any reasonable standards which will allow for analysis and valid interpretation of the data. Inadequacies and/or irregularities in this study include:

- multiple transfers of the study animals between research centers while the study was in progress,
- group housing of test animals during transfer,
- inconsistencies in the reporting of the experimental

design (i.e., number of animals per test group, animal numbering [identification] system, and test drug dose),
d) irreconcilable differences in the mortality and survivorship data (i.e. there were too many animals alive at the conclusion of the study based on the initial group sizes and the reported mortality for each treatment group),
e) no body weight, clinical chemistry and hematology, or survivorship data is available for review from the on-study interval between 6 and 15 months,
f) all histopathology data from animals which died, were euthanized moribund, or sacrificed for interim analyses during the interval from 6-15 months have been lost from the study and are unavailable for interpretation of the experimental results.

4) A moderate to marked bilateral loss of retinal and choroidal blood perfusion with a resultant gray-white fundic appearance was noted in three female animals from the high dose group. Examinations were performed on 10 animals/sex from the high and control groups. This effect was also observed in the recently submitted rat carcinogenicity study (NDA 18-859, submission 050).

5) The histopathology data are inconclusive as regards the oncogenic potential of ribavirin. While there is some evidence of and increased incidence of pituitary adenomas in animals sacrificed at the termination of the study, these findings are not confirmed by data obtained from animals found dead or sacrificed moribund between months 15-24. Data regarding the incidence of mammary adenomas are unclear as this is a particularly common lesion observed in older rats.

**APPEARS THIS WAY
ON ORIGINAL**